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PRELIMINARY BEHAVIORAL ASSESSMENT OF HABITUATION TO THE INSECTI--ETC(U)
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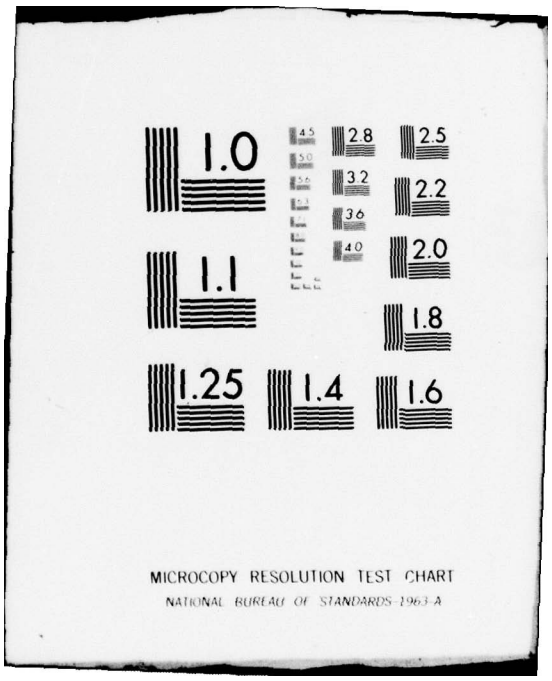
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# UNITED STATES ARMY ENVIRONMENTAL HYGIENE AGENCY

## ABERDEEN PROVING GROUND, MD 21010



AEHA

PRELIMINARY BEHAVIORAL ASSESSMENT OF
HABITUATION TO THE INSECTICIDE PERMETHRIN
STUDY NUMBER 75-51-0026-79
AUGUST - OCTOBER 1978



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Behavioral observations of immature male rats showed that they habituated to inhalation of permethrin aerosols. Habituation was carried out by exposing three groups of five rats to aerosols of permethrin for 21 days at 500 mg/m$^3$, followed immediately by exposure to 1,000 mg/m$^3$ for an additional 21 days. Three groups of five rats each were not exposed but lived in identical housing. At the end of this period, all rats (both habituated and control) were given one 4-hour exposure to 5,000 mg/m$^3$. At the end of the 500 and 1,000 mg/m$^3$ habituation

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period, there were no differences in retention of avoidance training. There were also no differences in the ability of five other exposed and five control rats to learn the same task. However, after the 5,000 mg/m$^3$ exposure, a third unhabituated group showed significantly worse retention than the simultaneously exposed habituated rats and did significantly worse than on preexposure performances. These nonhabituated rats also showed decreases in coordination and balance and an increase in conflict behavior and tremors. No similar changes were shown by the habituated group.

DEPARTMENT OF THE ARMY
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010

CPT Sherman/jg/584-3627

HSE-LT/WP

8 MAY 1979

SUBJECT: Preliminary Behavioral Assessment of Habituation to the Insecticide Permethrin, Study No. 75-51-0026-79

Executive Secretary
Armed Forces Pest Control Board
Forest Glen Section, WRAMC
Washington, DC 20012

A summary of results and conclusions of the inclosed report follows:

a. Behavioral observations of immature male rats showed that they habituated to inhalation of permethrin aerosols.

b. Habituation was carried out by exposing three groups of five rats to aerosols of permethrin 6 hours per day, 5 days per week, for 21 days at 500 $mg/m^3$, followed immediately by exposure to 1,000 $mg/m^3$ for an additional 21 days. Three groups of five rats each were not exposed but lived in identical housing. One of these control groups was exposed only to room air in another inhalation chamber. At the end of this period, all rats (both habituated and control) were given one 4-hour exposure to 5,000 $mg/m^3$.

c. One exposed and one control group lived in a movement sensing apparatus throughout the study. The exposed rats showed visible tremors which, according to the movement sensor's output, gradually decreased through the night until normal activity levels were observed near morning. Tremors were greatest following exposures early in the week. No major differences were seen in the number of ambulatory or medium size movements.

d. Five exposed and five control rats were given avoidance training before exposures started. At the end of the 500 and 1,000 $mg/m^3$ habituation period, there were no differences in retention. There were also no differences in the ability of five other exposed and five control rats to learn the same task. However, after the 5,000 $mg/m^3$ exposure, a third unhabituated group showed significantly worse retention than the simultaneously exposed habituated rats and did significantly worse than on preexposure performances. These nonhabituated rats also showed decreases in coordination and balance and an increase in conflict behavior and tremors. No similar changes were shown by the habituated group.

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DEPARTMENT OF THE ARMY
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010

HSE-LT/WP

# PRELIMINARY BEHAVIORAL ASSESSMENT OF HABITUATION TO THE INSECTICIDE PERMETHRIN STUDY NUMBER 75-51-0026-79 AUGUST - OCTOBER 1978

1. AUTHORITY.

a. Memorandum of Understanding, Coordination of Biological and Toxicological Testing of Pesticides, between the US Army Environmental Hygiene Agency; the US Army Health Services Command; the Department of the Army, Office of The Surgeon General; the Armed Forces Pest Control Board; and the US Department of Agriculture, effective 23 January 1979.

b. Letter, AFPCB, Armed Forces Pest Control Board, 21 October 1975, subject: Request for Toxicological Evaluation.

c. Letter, AFPCB, Armed Forces Pest Control Board, 5 April 1977, subject: Request for Toxicological Evaluation.

2. REFERENCES.

a. Report, USAEHA-LT/WP, this Agency, Development of an Efficient Test System for Assessing Behavioral Effects of Exposure to Chemical Compounds, Study No. 51-051-73/75, November 1972 - November 1973 (AD 786519).

b. Report, HSE-LT/WP, this Agency, Preliminary Assessment of the Acute Toxicity of Malathion in Animals, Study No. 99-002-74/76, September 1973 - August 1975 (ADA 016146).

c. Report, HSE-LT/WP, this Agency, Behavioral and Biochemical Effects of Malathion, Study No. 51-051-73/76, October 1975 - April 1976.

d. Report, HSE-LT/WP, this Agency, Toxicological Evaluation of 3-(Phenoxyphenyl) Methyl(±) - Cis, Trans-3-(2,2-Dichloroethenyl)2,2-Dimethyl-cyclopropanecarboxylate (Permethrin), Study No. 51-0831-78, December 1975 - April 1977 (ADA 047284).

e. Report, HSE-LT/WP, this Agency, Subchronic Inhalation Toxicity of 3-(Phenoxyphenyl) Methyl - Cis, Trans-3-(2,2-Dichloroethenyl-2,2-Dimethyl-cyclopropanecarboxylate (Permethrin), Study No. 51-0026-78, May 1978 - October 1978 (in preparation).

3. PURPOSE. To evaluate the behavioral changes produced by inhalation exposure to permethrin.

4. BACKGROUND.

a. Observations made during a study recently carried out by Toxicology Division (reference 2e above) indicated that rats exposed to permethrin showed severe tremors for the first few days of inhalation which gradually decreased and then disappeared as the number of days of exposure increased. A study was designed to assess possible rodent adaptation to permethrin (2e above). Adjunctive behavioral measures appeared to be warranted as the major sign prompting the study (tremors) was indicative of a Central Nervous System (CNS) effect. While the study was being carried out, thirty of the animals were given behavioral tests in addition to their basic role in the study in order to provide a preliminary assessment of any CNS changes occurring which might not be ascertained by standard chemical, physical or visual observation methods.

b. Habituation is defined as the waning of responsiveness to repetitions of a constant stimulus.* Thus, the reported exposures to almost identical doses of permethrin constitute repetitive physical stimuli and the slow decrease in extent of tremors is indicative of habituation to the insecticide.

5. PROCEDURE.

a. Animals. Thirty male Sprague-Dawley Albino rats (Wistar-derived strain) from an ongoing study, fully described elsewhere (reference 2e above), participated in behavioral tests and observations. The rats in the ongoing study were obtained from the AEHA colony and had a mean weight of 100 gm for the 60 males and 90 gm for the 60 females. They were housed in experimental groups of five and had free access to food and water except during inhalation exposures.

b. Permethrin. Permethrin is 3-(Phenoxyphenyl methyl - cis, Trans-3-(2,2-dichloroethenyl)-2,2-Dimethylcyclopropanecarboxylate. The chemical was produced and supplied by S.B. Penick & Co., Lyndhurst, NJ, as SBP-1513 (lot number RAX-6) in 1978.

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* Marler, P. and Hamilton, W., (1966), Mechanisms of Animal Behavior, John Wiley Publishers, New York City, NY.

c. Exposure Sequence. Three groups (a total of 15 rats) were exposed to 500 mg/m$^3$ aerosols of permethrin for 6 hours per day, 5 days per week, in a 200 liter dynamic flow inhalation chamber for 21 days. These animals were then exposed to a 1000 mg/m$^3$ concentration of aerosol for an additional 21 days. Control rats were kept under identical conditions, and, whenever rats were exposed, one group of controls (5 rats) was placed in an inhalation chamber identical to the chamber in which the exposed rats were run. The following day all rats (both habituated and controls) were exposed to a 4-hour 5,000 mg/m$^3$ aerosol. The basic experimental design and procedure are illustrated in the Figure.

d. Behavioral Measures. The behavioral measures evaluated were:

(1) Long Term Group Acitivity, Social Interaction and Motor Function. Disturbances in group activity and activity rhythms are common results of toxicological insult. One group of five exposed and one of five control rats lived in movement sensor cages for the duration of the study. This control group was always placed in an inactive inhalation chamber when the habituation group was exposed. The monitor has two plastic 45 X 24 X 19 cm cages suspended from central pivots. It independently records small movements such as tremors, medium movements such as rearing, and large movements such as walking. The monitor was programmed to printout totals of all three activity levels at 10-minute intervals between 1630 hours and 0800 hours every night of the study. Automatic recordings were correlated with visual observations of activity and behavior on a daily basis for 15 minutes after the rats were replaced on the sensor. These observations were videotaped for 1 minute each day. The taped sequence showed the entire cage for an overview of social interactions and movement. The camera then zoomed in for closeups of individuals to record small movements and tremors. Both exposed and control animals were taped. This permitted immediate contrast of behaviors shown on different days.

(2) Balance and Coordination. One day after the control and habituated rats living in the activity sensor received the 5,000 mg/m$^3$ dose, each individual was placed in the center of a horizontal rough wood beam 3 cm wide, 2 m long and 1.5 m off the floor. The rat's ability to walk along the beam was measured by recording whether or not the animal fell off before reaching one end. If the animal did not move by 15 seconds after placement, his hind quarters were gently prodded with sufficient pressure to cause a reaction but not enough to directly alter his position or balance. Coordination was measured by placing the rat on the side of a vertical metal grid with 1.5 cm squares and observing his mobility. The grid was 75 cms square.

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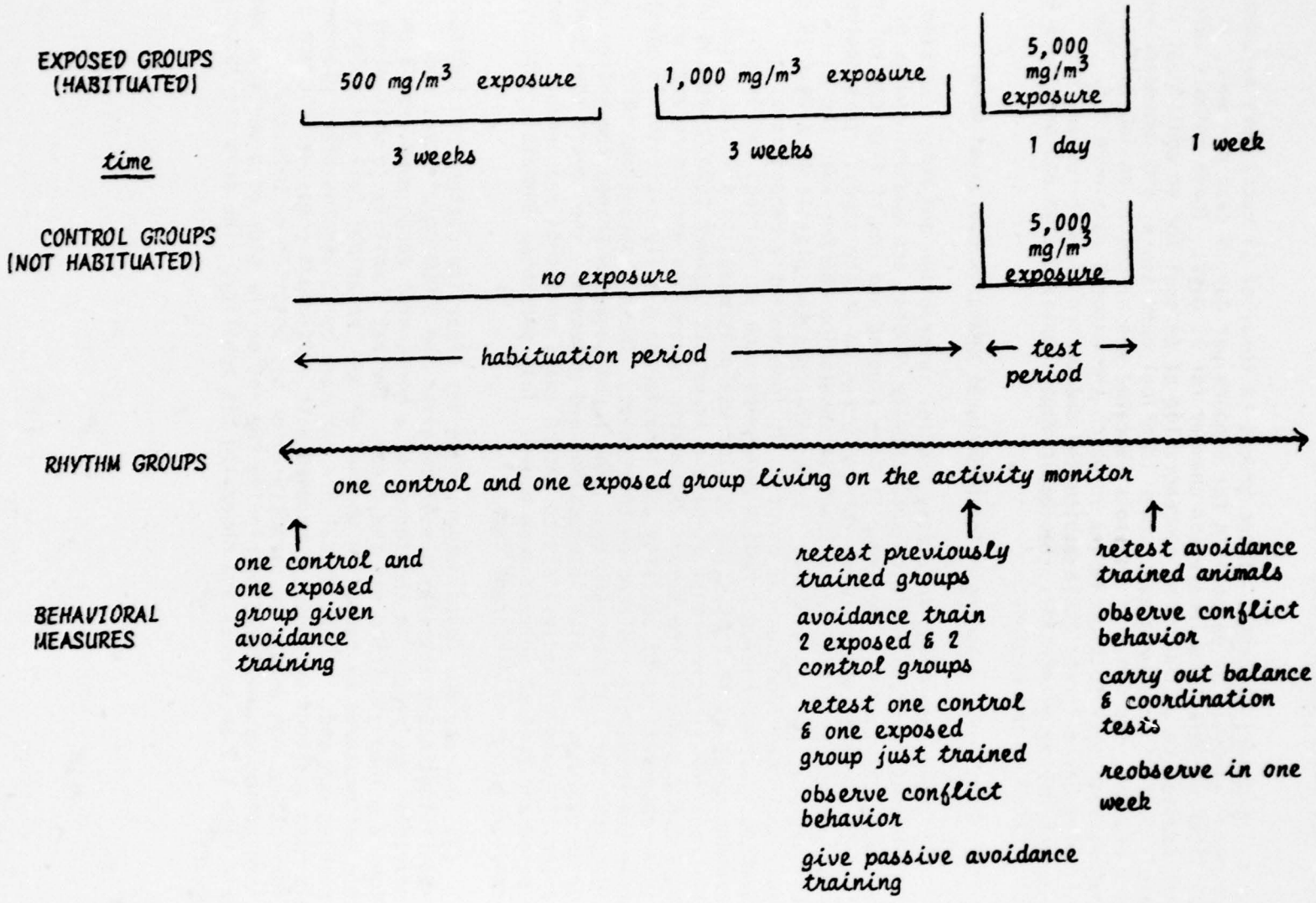


FIGURE. PROCEDURE

(3) Conflict Behavior/Aggressiveness. Changes in aggressiveness were measured by 15-minute observations of the groups of rats living in the activity monitor. Threat, attack and submission postures are highly stereotyped among rats and physical conflicts are readily recognized. Once a group has stabilized, aggressive displays are rare and physical conflicts almost nonexistent. The most likely time for conflicts to occur is just after the rats are replaced in their home cage after an absence of some hours. Thus, the 15 minute observations were made when the rats were replaced in their movement sensor cages after 4 to 6 hours of isolation in the inhalation chambers.

(4) Quick Avoidance (Post exposure retention of a task learned prior to exposure). Quick avoidance training was given a day before exposure and retention was measured a day afterwards. The method was identical to that used in earlier studies at the AEHA (references 2a, b and c above). The apparatus consisted of a translucent plexiglass "start" box 9 X 9 X 21 cm with a hinged lid and an 8 X 8 cm doorway at the end of one side. The doorway opened into a 28 X 26 X 24 cm "safe" compartment. The start box contained a grid floor through which the rat could receive a mild scrambled shock at 1.75 milliamperes. This was sufficient to cause the rat to lift his feet but not jump, squeek or convulse. Preexposure training consisted of four trials. The rat was placed into the start box and, when the lid was closed, a 1000 Hz 80 decibel tone sounded as the shock was turned on. The rat had to leave the box through the doorway to terminate the shock. The trials were 5 minutes apart and were followed by two test trials which were also 5 minutes apart. The test trials differed from training trials in that the rat could avoid the shock by leaving the start box within 20 seconds of insertion. The rats were retested after exposure and the number of seconds between insertion and exit was used as the measure of retention.

(5) Passive Avoidance/Post Exposure Learning. One 3-minute passive avoidance training session was given after the long term exposure (habituation) period to test changes in learning ability. The apparatus consisted of a plastic 30 X 21 cm box with a grid floor. A train of mild shocks alternating 1 second on with 1 second off, and similar in intensity to those described above, was given whenever the rat moved away from the far left side of the box. Thus, the animal had only to remain in the "safe" area to avoid shocks. This procedure eliminates confounding variables due to changes in motor ability.

6. RESULTS.

a. Activity and Motor Control.

(1) Tremors. Videotaped observations of exposed rats clearly indicated an adaptive pattern. They showed tremors on the first day of each week's exposure which generally decreased in intensity through the week and were

generally proportionate to the average daily dose. The activity monitor did not show significant differences in tremors between control and exposed rats by the end of the habituation period. The controls showed a significantly higher (Alpha less than 0.005 with a one tailed "t") level of tremors after their 5,000 mg/m$^3$ exposure than did the similarly exposed habituated rats. Numerical data from 1 day prior to exposure, 1 day and 1 week after exposure are presented in Table 1a.

(2) Medium and Large Motor Movements. These magnitudes of activity tend to reflect exploration, sniffing, social interactions, walking, etc. The activity monitor did not detect significant differences between the controls and exposed groups during the habituation period. However, after the 5,000 mg/m$^3$ dose, the nonhabituated controls showed significantly more activity than the habituated group. These data are summarized in Tables 1b and c.

(3) Reactivity. Videotaped observations of exposed and control rats showed that, during the habituation period, exposed rats reacted much more vigorously to hand claps than unexposed controls. The exposed rats all jumped violently after the clap but there was little hyperresponsiveness to the adjacent movements of other animals. This behavior decreased as the habituation process progressed. Unexposed controls generally showed typical mild orienting responses to the hand clap. However, after the 5,000 mg/m$^3$ exposure, the nonhabituated controls showed even more reactivity to hand claps than exposed animals originally did and were also hyperreactive to the movements of nearby animals for several days.

b. Balance and Coordination. After the 5,000 mg/m$^3$ exposure, 80 percent of the nonhabituated rats fell off the balance beam while only 20 percent of the habituated animals did. None of the nonhabituated rats were able to maneuver easily on the vertical grid and two fell off. All of the habituated rats moved comfortably. Thus, the balance and coordination of the habituated animals were not as affected as that of the nonhabituated ones.

c. Conflicts and Social Interactions. During the habituation period, both control and exposed rats showed highly stable social orders (e.g., dominance hierarchy) and activity. Thus stereotyped threat-submissiveness postures were rare and actual physical conflicts were virtually absent during the observational periods. Social interaction appeared normal for both groups. After the 5,000 mg/m$^3$ exposure, the habituated rats showed no increase in conflicts or change in social behavior. The nonhabituated controls showed a complete disruption of all normal patterns of interanimal behavior. Conflict was actually continuous in the cage during observation periods. Stereotyped threat postures were enacted but submissive postures were not held so physical conflicts frequently ensued. Results of an encounter did not serve to establish a dominance hierarchy (pecking order) so the same two rats would go through the entire process whenever they met. As there were five animals in the cage, meetings were continuous, and, thus, so

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TABLE 1. DATA FROM THE ACTIVITY MONITOR

Behavior of groups living on the monitor. "A" & "B" represent half hour readings taken one hour apart starting when the rats were replaced in the monitor. The data is shown for the mean and standard deviation. A "*" indicates that the animals were sleeping when the reading was taken. "DIFF" indicates statistically significant differences with the chances of the groups being the same less than one in 5,000 using at one tailed "t" test.

a. VIBRATIONS

| | | 1 day before 5,000 exposure | 1 day after 5,000 exposure | | 1 week after 5,000 exposure |
|---|---|---|---|---|---|
| NOT HABITUATED | A | 484 (164) | 3,394 (788) | DIFF | 201 (32) |
| | B | * | 2,314 (447) | | * |
| HABITUATED | A | 337 (136) | 188 (79) | | 123 (8) |
| | B | * | * | | * |

b. MEDIUM MOVEMENTS

| | | | | | |
|---|---|---|---|---|---|
| NOT HABITUATED | A | 106 (65) | 554 (90) | DIFF | 350 (92) DIFF |
| | B | * | 369 (82) | | * |
| HABITUATED | A | 56 (37) | 9 (3) | | 22 (14) |
| | B | * | * | | * |

c. LARGE MOVEMENTS

| | | | | | |
|---|---|---|---|---|---|
| NOT HABITUATED | A | 90 (37) | 294 (71) | DIFF | 189 (44) |
| | B | * | 208 (55) | | * |
| HABITUATED | A | 60 (27) | 121 (14) | | 280 (113) |
| | B | * | * | | * |

TABLE 2. QUICK AVOIDANCE RETENTION.

| | | BEFORE HABITUATION PERIOD | AFTER HABITUATION PERIOD | AFTER 5,000 MG/M3 EXPOSURE | |
|---|---|---|---|---|---|
| RATS FROM ACTIVITY MONITOR | NOT HABITUATED | | 3.5 (7.6) | *retest* 13.6 (10.7) | DIFF |
| | HABITUATED | | 4.6 (8.5) | 2.0 (2.8) | |
| RATS FROM MAIN ROOM (NOT PRETRAINED) | NOT HABITUATED | | 2.2 (7.1) | | |
| | HABITUATED | | 1.0 (1.8) | | |
| RATS FROM MAIN ROOM (PRETRAINED) | NOT HABITUATED | 4.5 (5.4) | *retest* 0.8 (1.2) | | |
| | HABITUATED | 4.4 (5.4) | 0.3 (0.7) | | |

*DIFF = significantly different at greater than 0.005 (One tailed "t").*
*retest = second time the group was run through the test process described in the text.*
*The numbers represent group means in seconds and the standard deviation.*

TABLE 3. PASSIVE AVOIDANCE TRAINING (POST EXPOSURE LEARNING)

| | | # SHOCKS | # SECONDS SHOCKED | LAST SHOCK AT SEC # |
|---|---|---|---|---|
| RATS FROM ACTIVITY SENSOR | NOT HABITUATED | 4.0 (1.1) | 6.8 (5.2) | 78 (42) |
| | HABITUATED | 8.4 (2.2) | 7.2 (3.6) | 84 (40) |
| RATS FROM MAIN ROOM | NOT HABITUATED | 4.6 (2.7) | 3.4 (3.8) | 32 (32) |
| | HABITUATED | 4.5 (0.9) | 8.0 (6.1) | 44 (26) |

*There were no significant differences between groups.*

were confrontations. This behavior persisted for several days then gradually decreased to normal levels so no conflicts were observed at the end of 1 week.

d. Retention of Preexposure Learning (Quick Avoidance). There were no differences between habituated and control rats in the number of seconds required to leave the start box in quick avoidance tests either for animals trained before the habituation period started and retested at its end or for animals trained and retested after the habituation period. There were significant differences (greater than 0.005 with one tailed "t"s) between habituated and nonhabituated animals trained prior to the 5,000 $mg/m^3$ exposure and retested a day afterwards. The nonhabituated animals required a mean of 13.6 seconds to leave while the habituated animals required only 2.0 seconds to leave. These data are summarized in Table 2.

e. Post Habituation Learning/Passive Avoidance. There were no differences in the ability of nonhabituated and habituated rats to learn a new task (passive avoidance) at the end of the habituation period. These data are summarized in Table 3.

f. Water Consumption. The exposed rats consistently consumed about one third more water than controls kept in the nonexposure inhalation chamber. Temperature, humidity and animal density were similar in both chambers.

7. CONCLUSION.

a. The results show that after more than a month of exposure to varying relatively low doses of permethrin, retention of a learned task is not effected. The habituated group's retention is still not effected when exposed to a relatively high dose. The same high dose does adversely effect retention among control rats not previously habituated to permethrin. The high dose produced significantly more tremors and heightened aggressiveness among nonhabituated than among habituated rats. The dose also adversely effected the balance and coordination of the nonhabituated animals but not those of the habituated ones. Thus, rats do show behavioral habituation to permethrin.

b. It must be noted that the groups consisted of only five animals each and no females were tested. So, although all differences reported were significant beyond the 0.005 level (one tailed "t" tests), repetition with larger groups or females may give different results. This study does indicate that a larger evaluation using more frequent behavioral measures and larger groups would be likely to produce significant results.

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